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(54) NON-ANIMAL LIPASE PREPARATIONS WITH IMPROVED ACTIVITY

(71) We, DEUTSCHE GOLD-UND SILBER-SCHEIDEANSTALT VORMALS ROESSLER a body corporate organised under the laws of Germany of 9 Weissfrauenstrasse, 6 Frankfurt Main 1, Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to non-animal lipase preparations with improved activity. According to a preferred embodiment it relates to improved pharmaceutical preparations containing lipase of non-animal origin.

Lipase preparations are of a commercial significance and may be used for example for splitting fats. Lipase preparations can also be used for bating in preparatory tanning.

For the purposes of standardisation, the commercial products are mixed with inert substances, such as for example sugar, starch, kieselguhr, etc. These additives have to be regarded as integral constituents of the preparations. Now, it has been found that lipase frequently undergoes a considerable reduction in its activity under the effect of additives such as these, particularly where they are surface-active substances.

It has now been found that this reduction in activity occurs to only a very minimal extent, if at all, in the preparations containing lipase of non-animal origin according to the invention.

The present invention provides an enzyme preparation containing a lipase of non-animal origin and, as stabiliser, 10 to 50 parts by weight (based on 1 part by weight of lipase) of a mixture of the following constituents: 40 to 90% of lactose; 8 to 50% of whey protein; 0.1 to 7% of milk fat; 0.1 to 10% of whey minerals and 0.1 to 5% of water (%=% by weight).

A composition containing as stabiliser a whey product of the following composition shows a good stabilising effect:

49 to 51% of lactose
36 to 40% of whey protein
3 to 5% of milk fat
6 to 7% of whey minerals
2 to 3% of water.

According to a further embodiment the invention provides an enzyme preparation containing a lipase of non-animal origin and, as stabiliser, 10 to 50 parts by weight and preferably from 20 to 40 parts by weight (based on 1 part by weight of lipase) of a mixture of the following constituents: 70 to 90% and preferably 82 to 86% of lactose; 8 to 20% and preferably 11 to 13% of whey protein; 0.1 to 3% and preferably 0.2 to 0.5% of milk fat; 0.1 to 5% and preferably 1 to 2% of whey minerals and 0.1 to 3% and preferably 0.5 to 2% of water (%=% by weight).

The invention also provides a process for the production of the enzyme preparations containing lipase of non-animal origin and having improved activity defined above which comprises mixing the constituents defined. The invention also provides a process for stabilising an enzyme preparation containing lipase of non-animal origin which comprises incorporating therein the stabiliser mixture defined.

In the context of the invention, whey protein or serum protein are the proteins which are present in the unheated milk after precipitation of the casein (by electrolytes, especially hydrogen ions or rennet). This milk serum protein has the following composition for example: 55 to 65% of β_1 and β_2-

lactoglobulin, 13 to 22% of α-lactalbumin, 16 to 25% of immune globulins and 3 to 4% of serum albumin.

Milk fat consists of the triglycerides of saturated and unsaturated fatty acids. The fatty acid composition (in % by weight) is for example as follows: butyric acid 2 to 8, preferably 3.6 to 5.5; caproic acid 1 to 3, preferably 1.5 to 2; caprylic acid 0.1 to 2, preferably 0.5 to 1; capric acid 0.1 to 5, preferably 0.3 to 2.5; lauric acid 1 to 5, preferably 2.0 to 2.6; myristic acid 5 to 15, preferably 9.5 to 12; palmitic acid 25 to 40, preferably 28 to 38; stearic acid 1 to 15, preferably 1.5 to 10; saturated acids over C_{16} 1 to 4, preferably 2 to 3; decanoic acid 0.05 to 0.5; dodecanoic acid 0.05 to 0.5; tetradecanoic acid 0.1 to 3; hexadecanoic acid 1 to 7; octadecanoic acid 20 to 40, preferably 25 to 35; octadecadienoic acid 1 to 5; unsaturated acids over C_{18} 1 to 3.

In the context of the invention, whey minerals are a mixture of phosphates, citrates, chlorides, sulphates and carbonates of sodium, potassium, calcium, magnesium and, in traces, iron. The content of the metals in the salt mixture in % by weight is for example as follows: sodium 2 to 8, preferably 4 to 5% by weight; potassium 10 to 30, preferably 15 to 20% by weight; calcium 2 to 30, preferably 4 to 15% by weight; magnesium 0.5 to 3, preferably 0.8 to 1.5% by weight; iron 0.01 to 0.1, preferably 0.02 to 0.08% by weight. For every part by weight of magnesium, the mixture contains for example from 3 to 8 and preferably from 5 to 7 parts by weight of sodium, from 2 to 15 and preferably from 4 to 8 parts by weight of calcium and from 15 to 30 and preferably from 18 to 25 parts by weight of potassium.

The content of acids in % by weight in the salt mixture is for example as follows: phosphate anion (PO_4^{3-}) 10 to 50, preferably 15 to 25% by weight; citrate anion 10 to 30, preferably 15 to 25% by weight; chloride anion 10 to 20, preferably 12 to 16% by weight; sulphate anion 2 to 8, preferably 3 to 6% by weight; carbonate anion 2 to 15, preferably 5 to 10% by weight; based on 1 g of sulphate anion, the mixture contains for example from 2 to 5 parts by weight and preferably from 2.5 to 3 parts by weight of chloride anion, from 3 to 10 and preferably from 4 to 6 parts by weight of phosphate anion, from 3 to 10 and preferably from 4 to 6 parts by weight of citrate anion and from 0.5 to 2 and preferably from 0.8 to 1.5 parts by weight of carbonate anion.

The stabiliser preferably used in accordance with the invention is sweet whey powder employed in a quantity of from 10 to 50 parts by weight and more especially in a quantity of from 20 to 40 parts by weight (based on 1 part by weight of lipase). Sweet whey powder is a commercially available product and is obtained for example by spray drying whey (sweet whey); *cf.* Ullmanns Encyclopadie der technischen Chemie, 12th Vol. (1960), page 518; see also E. Wegelin, Netherlands Milk and Dairy Journal 5, 263/73 (1951); S. G. Wiechers, ebenda 6, 127/36 (1952); S. G. Wiechers, A. H. Willem, Nederlandse Centrale Org. voor Toegepast - Natuurwetenschappelijk Onderzoek, German Patent No. 819,194, 1949; Food 23, 166/71 (1954).

The sweet whey powder used is a mixture of 70 to 90% and preferably 82 to 86% of lactose, 8 to 20% and preferably 11 to 13% of whey protein, 0.1 to 3% and preferably 0.2 to 0.5% of milk fat, 0.1 to 5% and preferably 1 to 2% of whey minerals and 0.1 to 3% and preferably 0.5 to 2% of water (%=% by weight).

The lipase to be stabilised is of non-animal origin. For example, the lipase may be from plants, for example from castor beans, or lipase from microorganisms, for example from fungi such as *Rhizopus arrhizus, Rhizopus nigricans, Rhizopus oryzae, Rhizopus delemar, Aspergillus* strains such as *Aspergillus niger, Aspergillus oryzae* or *Welchia perfringens, Mycotorula lipolytica, Candida cyclindracea, Geotrichum candidum.*

The lipases in question may be obtained in known manner, as described for example in Ullmanns Encyklopadie der technischen Chemie, Vol. 7)1956), pages 391—397 and 406—411, or in Bulletin de la Societe de Chimie Biologique 1966, 48, No. 6, pages 747—770 and 1968, 50, No. 11, pages 2179—2182. For example, a fungus lipase is obtained as follows in accordance with Bulletin de la Societe de Chimie Biologique 1966, 48, No. 6, pages 747 *et seq.* The filtrate obtained after separation of the mycelium from cultures of fungus spores is concentrated *in vacuo* at 30°C and centrifuged and, after dilution, the enzyme is precipitated from the upper layer with acetone at 0°C and dried *in vacuo*. For further purification, it is suspended in water and centrifuged, SO_4-ions are precipitated by the addition of barium chloride, the enzyme is reprecipitated with acetone and the residue thus obtained after dissolution in distilled water is introduced into a calcium-charged XE64 exchanger column at pH 4.7 and eluted with a calcium acetate solution at pH 5.7. After adjustment to pH 6 with dilute ammonia, the enzyme is precipitated from the eluate with acetone and the product thus obtained is rechromatographed in a G25 Sepphadex ("Sephadex" is a Registered Trade Mark) column by suspension in demineralised water. The lipase is obtained in powder form from the resulting eluate by freeze drying (−70°C).

The stabilising effect is particularly pronounced in the case of a lipase from fungi of the *Rhizopus* genus, particularly *Rhizopus*

arrhizus. For example, a lipase having an activity of 8,800,000 units/g (as determined with an olive oil emulsion) is obtained by the above-mentioned process from Bull. Soc. Chim. Biol. 1966, pages 747 *et seq*. This lipase is a pure product and is primarily characterised in that it has two pH-optima, one at around pH 7 and the other at around pH 5. It behaves like a single protein both in paper electrophoresis, in polyacrylamide gel electrophoresis and also in chromatography on Sephadex columns and, in its mode of action, particularly resembles triglycerides of the pancreas lipase (for further properties, see Bull. Soc. Chim. Biol. 1966, 48, No. 6, pages 756—766). An increase in the activity of this lipase can be obtained by further purification in a G100 Sephadex column (in distilled water), after which a product having an activity of 11,000,000 units/g is obtained (see Bull. Soc. Chim. Biol. 1968, 50, No. 11, pages 2179—2182).

The stabiliser according to the invention improves the stability of lipase when it is combined and/or mixed with additives of the type normally used for commercial enzyme preparations, particularly those having surface-active properties, such as active alumina, aluminium hydroxide, aluminium oxide, highly disperse silica (Aerosil, the word "Aerosil" being a Registered Trade Mark), magnesium carbonate, aluminium salts (aluminium trisilicates, aluminium phosphates), dialkyl polysiloxanes, silica gel, kieselguhr and the like.

The expression "active alumina" applies to powder-form oxides, oxide hydrates, hydroxides and basic salts of aluminium which contain no less than 40% of Al_2O_3 (*cf.* Ullmanns Encyclopadie der technischen Chemie, 3rd Edition, Vol. 4, pages 545/546 and Vol. 13, page 356). In particular, the expression "active alumina" applies to an aluminium hydroxide gel obtained by precipitating aluminium salt solutions (for example sulphate solutions) with ammonium carbonate or sodium carbonate and drying the filter cake (Al_2O_3-content no less than 47%, preferably 50 to 60%). The pH-value of a 4% (weight/volume) suspension in CO_2-free water should not exceed 10.0 Aluminium hydroxide gels such as these are commercially available for example under the name "Teg".

The highly disperse silica is a silica obtained by the hydrolysis of silicon tetrachloride in a hydrogen flame (Aerosil). A silica such as this has the following characteristics for example: BET-surface (m^2/g): 50 to 225, preferably 120 to 225 or more preferably 170 to 225; average primary particle daimeter in millimicrons: 12 to 30, preferably 12 to 16; apparent density (normal product) in g/litre: approx. 60; bulking value (normal product according to DIN 53 194) in ml/100 g: 1500 to 2000, preferably 1700 to 2000; pH-value (according to DIN 53 200) in 4% aqueous dispersion: 3.5 to 4.3, preferably 3.6 to 4.3.

The dialkyl polysiloxanes are known and are commercially available, being obtained by the usual processes, for example by the polymerisation of silicones or by the hydrolysis and chemical condensation of one or more hydrolysable silicone compounds corresponding to the general formula R_2SiX_2, where R is a lower alkyl group containing from 1 to 3 carbon atoms, preferably from 1 to 2 carbon atoms, and X is a halogen atom (for example chlorine) or a lower alkoxy group. Examples of starting compounds such as these are dimethyl dichlorosilane, diethyl dichlorosilane, dimethyl diethoxy silane, methylethyl dichlorosilane, dibutyl dichlorosilane, dihexyl chlorosilane, ethyl butyl diethoxy silane and the like.

For example, the hydrolysable silicone is reacted with water under defined conditions to give a polysiloxane having the required viscosity. The preferred polysiloxane is is dimethyl polysiloxane.

For example, the material in question may be a material having the free name of Simethicon. This material consists essentially of dimethyl polysiloxane and 4 to 4.5% by weight of a silicon dioxide aerogel. For example, the silicone fluxing agent of this mixture, which is manufactured by the Dow Corning Chemical Company, has a molecular weight of from 14,000 to 21,000, a silicon content of from 37.3 to 38.5%, a viscosity at 25°C of from 300 to 600 centistokes (cs), a density at 25°C of from 0.965 to 0.970 and a refractive index n_D^{25} of 1.403 ± 0.002.

The average molecular weight of the dialkyl polysiloxanes is preferably between 14,000 and 30,000, for example approximately 24,000. The viscosity of the dialkyl polysiloxanes may amount for example to between 900 and 1100 cP (25°C); it is preferably in the range from 950 to 1050 cP. The proportion of low polymers (up to a molecular weight of 700) should be small, amounting to less than 0.5%.

Silica gel is an active silica which consists for example of particles from 5 mμ to 4 mm in diameter. The total inner surface area of 1 g of silica gel may amount for example to between 400 and 800 m^2 (*cf.* Rompp Chemie-Lexikon, 1966, Vol. IV, 5915—5916; Ullmanns Encyclopadie der Technischen Chemie, Vol. 15 (1964), pages 716—732). The particle diameter is preferably between 5 and 100 mμ and preferably between 10 and 50 mμ. The silicon dioxide used has a surface of, for example, from 100 to 250 m^2/g and preferably from 150 to 200 m^2/g. The water content is for example from 0.5 to 2% and preferably from 0.7 to 1.5%. The silicon dioxide may be produced by known methods, for example by the action of

sulphuric acid on water glass or by the hydrolysis of $SiCl_4$ (Degussa process; *cf.* for example US Patent No. 3,086,851, DAS No. 1,163,784, DAS No. 1,210,421, DAS No. 1,150,955).

In addition, the lipase-containing preparations may contain other enzymes, for example proteolytically active enzymes and amylases (for example proteases and amylases such as are present for example in enzyme concentrates from *Aspergillus* strains, such as *Aspergillus oryzae* or even *Aspergillus parastiticus*).

The preparations are produced by mixing the lipase of non-animal origin with the stabiliser according to the invention and optionally, with other standard additives in the mixers and homogenisers normally used for this purpose (for example tumble mixers, forced circulation mixers).

This aspect of the invention is illustrated by the following Example:

EXAMPLE 1

1 part by weight of lipase is finely ground and mixed in a mortar with 20 parts by weight of aluminium hydroxide gel (dried) and 30 parts of sweet whey powder (temperature 20°C).

If the sweet whey powder is left out and replaced by 30 parts by weight of glucose, a loss of activity of around 40% is observed after mixing by comparison with the mixture containing the sweet whey powder.

As previously mentioned the invention also relates to improved pharmaceutical preparations containing lipase of non-animal origin.

The use of ferment preparations for the treatment of digestive disorders has long been known. Perorally administered preparations preferably containing all three ferment systems, the protein-splitting enzymes (proteases), the carbohydrate-splitting enzymes (amylases) and the fat-splitting enzymes (lipases) are used for this purpose. These enzymes are normally obtained from the organs of animals. However, one disadvantage of these animal enzymes is that they are rapidly destroyed in the acid stomach medium and, in addition, can only develop their effect in the intestine. This applies in particular to lipase and amylase.

By contrast, enzymes of non-animal origin, for example from plants and microorganisms, are much more stable to acids. They undergo hardly any reduction in activity on passing through the stomach and the upper sections of the intestine. In addition, it is possible with enzymes such as these to initiate the digestive process in the stomach itself.

In many cases, therefore, enzymes of non-animal origin are preferable by virtue of their wider pH-activity range to the animal enzymes with their narrow pH-activity range.

On the other hand, however, lipase of non-animal origin shows little stability and soon loses its activity. For example, it has been found that tablets produced in the usual way from fungus lipase (lipase from *Rhizopus arrhizus*) with addition of such auxiliaries as lactose, aluminium hydroxide, dimethyl polysiloxane (activated with silica gel), sorbitol, highly disperse silica and talcum, show a loss of lipolytic activity of as much as 50% immediately after their production. Replacing the above-mentioned additives by other additives of the type normally used for galenic purposes does nothing to change this loss of activity.

It has now been found that the stability of lipase of non-animal origin can be distinctly improved in the lipase-containing pharmaceutical preparations according to the invention.

The present invention provides a pharmaceutical preparation containing a lipase of non-animal origin and from 10 to 50 parts by weight (based on 1 part by weight of the lipase) of a mixture of the following constituents: 40 to 90% of lactose; 8 to 50% of whey protein; 0.1 to 7% of milk fat; 0.1 to 10% of whey minerals and 0.1 to 5% of water (%=% by weight).

The pharmaceutical preparation containing as stabiliser a whey product of the following composition shows a good stabilising effect:

49 to 51% of lactose
36 to 40% of whey protein
3 to 5% of milk fat.
6 to 7% of whey minerals
2 to 3% of water.

According to a further embodiment the invention provides a pharmaceutical preparation containing a lipase of non-animal origin and from 10 to 50 parts by weight and preferably from 20 to 40 parts by weight (based on 1 part by weight of the lipase) of a mixture composed of the following constituents: 70 to 90% and preferably 82 to 86% of lactose; 8 to 20% and preferably 11 to 13% of whey protein; 0.1 to 3% and preferably 0.2 to 0.5% of milk fat; 0.1 to 5% and preferably 1 to 2% of whey minerals and 0.1 to 3% and preferably 0.5 to 2% of water (%=% by weight).

The invention also provides a process for the production of the pharmaceutical preparation defined above which comprises mixing a lipase of non-animal origin with the stabiliser defined above. The invention also provides a process for stabilising a pharmaceutical preparation containing lipase of non-animal origin which comprises incorporating therein the stabiliser mixture defined.

The stabiliser preferably used in accordance with this aspect of the invention is sweet whey powder employed in a quantity of from 10 to 50 parts by weight and more particularly in a quantity of from 20 to 40

parts by weight (based on a tablet weight of 100 parts by weight).

A lipase of non-animal origin is stabilised in accordance with this aspect of the invention. The lipase in question is in particular lipase from plants, for example from castor beans, or lipase from microorganisms, for example from fungi such as *Rhizopus arrhizus, Rhizopus nigricans, Rhizopus oryzae, Rhizopus delemar, Aspergillus* strains such as *Aspergillus niger, Aspergillus oryzae* or *Welchia perfringens, Mycotorula lipolytica, Candida cylindracea, Geotrichum candidum.*

Based on a weight of the preparation of 100 parts by weight, they generally contain from 0.5 to 5 parts by weight, preferably from 0.8 to 2 parts by weight and, more especially from 0.8 to 1.5 parts by weight of lipase. It is preferred to use from 30 to 40 parts by weight of whey powder per part by weight of lipase.

The lipases are obtained in known manner, as described above.

It is preferred to use a lipase from fungi of the *Rhizopus* genus, particularly *Rhizopus arrhizus*. A lipase having an activity of 8,800,000 units/g (as determined with an olive oil emulsion) is obtained for example by the above-mentioned process described in Bull. Soc. Chim. Biol. 1966, pages 747 *et seq*. This lipase is a pure product and is primarily characterised in that it has two pH optima, one at around pH 7 and the other at around pH 3.5. It behaves like a single protein both in paper electrophoresis, in polyacrylamide electrophoresis and also in chromatography in Sephadex columns and resembles in particular triglycerides of the pancreas lipase in its mode of action (for further properties, see Bull. Soc. Chim. Biol. 1966, 48, No. 6, pages 756—766). An increase in the activity of this lipase can be obtained by further purification in a G 100 Sephadex column (in distilled water), after which a product having an activity of 11,000,000 units/g is obtained (*cf.* Bull. Soc. Chim. Biol. 1968, 50 No. 11, pages 2179—2182).

In particular, the stabiliser according to the invention produces an improvement in the stability of lipase when it is combined and/or mixed with at least one other pharmaceutical auxiliary or additive, particularly with those having surface-active properties, such as aluminium hydroxide, aluminium hydroxide gel, aluminium oxide (*cf.* H. P. Fiedler, Lexikon der Hilfsstoffe fur Pharmazie, Kosmetik und angrenzende Gebiete, 1971, pages 43 and 44), Aerosil, magnesium carbonate, aluminium salts (aluminium trisilicates, aluminium phosphates).

In addition, the lipase-containing medicaments according to the invention may contain further enzymes, particularly proteolytically active fungus enzymes and amylases. Preferred enzymes of this type are proteases and amylases of the type present for example in enzyme concentrates of *Aspergillus* strains, for example *Aspergillus oryzae* or even *Aspergillus parasiticus*.

The enzyme concentrate of *Aspergillus oryzae* is for example a multienzyme complex with a large proportion of acid proteinase, although it additionally contains considerable quantities of amylase, cellulase and protopectinases. Other enzymes with, in some cases, notable activity are peptidases (K. Lehmann, H. Uhlig, Hoppe Seylers Zeitschrift fur physiologische Chemie *350*, 99—104 (1969), neutral and alkaline proteinases, cellobiase, α-glycosidase and hemicellulases which split plant gum. The main components — acid proteinase, amylase, cellulase and pectinase — reach their optimum effect and stability in a pH range from 3.5 to 6. This is particularly favourable for pharmaceutical applications because the enzyme complex is able to develop its full activity in the stomach itself.

The acid proteinase of this enzyme concentrate has for example a pH optimum of 3—5, the neutral proteinase a pH optimum of 6—7 and the alkaline proteinase a pH optimum of 7—10 (*cf.* H. Sprecht, Die Proteinasen aus Aspergillus oryzae, Naturwissenschaften 44, 37—38 (1957).

Proteases and amylases or rather the mixed preparations which, in addition to other enzymes, contain large proportions of proteases and amylases are commercially available products and are obtained for example by the processes described in Ullmanns Encyclopadie der technischen Chemie, Vol. 7 (1956), pages 407—411 (the processes mentioned there are part of the disclosure). In particular fungus strains of *Aspergillus oryzae* are used.

The protease and/or amylase preparations are produced for example by the surface process by growing mold fungi (*Aspergillus oryzae*) on wheat bran nutrient substrates, extracting the enzymes formed after drying the substrate with water or buffer solution, precipitating them — optionally after concentration *in vacuo* (at the lowest possible temperatures) — with solvents such as acetone, ethanol, methanol, isopropanol, and so on or with ammonium sulphate, and then drying and pulverising the precipitated product. After standardisation with standard inert substances, such as sugar, glucose, lactose, starch, kieselguhr and so on, the products are ready for marketing. However, the preparations may also be produced by the submerse or deep-culture process in the usual way using, for example, *Aspergillus* strains, particularly *Aspergillus oryzae*.

It is also possible to add other active ingredients to the lipase preparations according to the invention.

The medicaments are produced in known manner, for which purpose the known and accepted pharmaceutical auxiliaries and other accepted excipients and diluents may be used.

Examples of excipients and additives of this kind are the substances recommended and specified in the following literature as additives for pharmacy, cosmetics and related fields: Ullmanns Encyklopadie der technischen Chemie, Vol. 4 (1953), pages 1 to 39; Journal of Pharmaceutical Sciencies, Vol. 52 (1963), pages 918 *et seq*, H. v. Czetsch - Lindenwald, Hilfsstoffe fur Pharmazie und angrenzende Gebiete; Pharm. Ind., No. 2, 1961, pages 72 *et seq;* Dr. H. P. Fiedler, Lexikon der Hilfsstoffe fur Pharmazie, Kosmetik und angrenzende Gebiete Cantor KG., Aulendorf (Wurtt), 1971.

Examples include gelatin, natural sugars such as cane sugar or lactose, lecithin, pectin, starch (for example corn starch), alginic acid, Tylose ("Tylose" is a Registered Trade Mark), talcum, lycopodium, silica (for example colloidal silica), cellulose, cellulose derivatives (for example cellulose ethers in which the cellulose hydroxy groups are partly etherified with lower saturated aliphatic alcohols and/or lower saturated aliphatic oxy alcohols, for example methyloxypropyl cellulose), stearates, magnesium and calcium salts of fatty acids with 12 to 22 carbon atoms, especially the saturated fatty acids (for example stearates), emulsifiers, oils and fats, especially vegetable oils and fats (for example peanut oil, castor oil, olive oil, sesame oil, cotton seed oil, corn oil, wheatgerm oil, sunflower seed oil, cod's liver oil, mono-, di- and triglycerides of saturated fatty acids $C_{12}H_{24}O_2$ to $C_{18}H_{36}O_2$ and mixtures thereof), pharmaceutically compatible monohydric or polyhydric alcohols and polyglycols, such as polyethylene glycols and derivatives thereof, esters of aliphatic saturated or unsaturated fatty acids (2 to 22 carbon atoms, especially 10 to 18 carbon atoms) with monohydric aliphatic alcohols (1 to 20 carbon atoms) or polyhydric alcohols, such as glycols, glycerol, diethylene glycol, pentaerythritol, sorbitol, mannitol and so on which, optionally, may even be etherified, benzylbenzoate, dioxolanes, glycerol formals, glycol furol, polyglycol ethers with C_1—C_{12}-alcohols, dimethyl acetamide, lactamides, lactates, ethyl carbonates, silicones (especially medium-viscosity dimethyl polysiloxanes), magnesium carbonate and the like.

Conventional solution promoters and emusifiers may be used in the preparation of the compositions. Examples of solution promoters and emulsifiers are polyvinyl pyrrolidone, sorbitan fatty acid esters, such as sorbitan trioleate, lecithin, acacia, tragacanth, polyoxyethylated sorbitan monooleate, polyoxyethylated fats, polyoxyethylated oleotriglycerides, linolised oleotriglycerides, polyethylene oxide condensation products of fatty alcohols, alkyl phenols of fatty acids. Polyoxyethylated in this context means that the substances in question contain polyoxyethylene chains with a degree of polymerisation of generally from 2 to 40, more especially from 10 to 20.

Polyoxyethylated substances of this kind may be obtained for example by reacting compounds containing hydroxyl groups (for example monoglycerides or diglycerides or unsaturated compounds such as, for example, those containing oleic acid radicals, with ethylene oxide (for example 40 moles of ethylene oxide per mole of glyceride).

Examples of oleotriglycerides are olive oil, peanut oil, castor oil, sesame oil, cotton seed oil and corn oil (see also Dr. H. P. Fiedler "Lexikon der Hilfsstoffe fur Pharmazie, Kosmetik und angrenzende Gebiete 1971, pages 191 to 195).

In addition, it is possible to add preservatives, stabilisers, buffers, for example calcium hydrogen phosphate, colloidal aluminium hydroxide, flavour correctants, anti-oxidants and complex formers (for example ethylene diaminotetraacetic acid) and the like. If desired, pH may also be adjusted to a certain range with physiologically compatible acids or buffers.

The compounds according to the invention are galenically handled by the usual standard methods. For example, lipase and other enzymes together with additives or excipients are thoroughly admixed by stirring or homogenisation (for example in colloid mills, ball mills), generally at low temperatures, for example 20°C, and pressed into tablet form in the usual way.

The lipase-containing preparations according to the invention are taken orally, preferably in the form of chewing tablets.

The dosage of the tablets normally amounts for example to between 1 and 2 tablets three times daily; the tablets may contain the quantities of enzyme and auxiliaries indicated in the Examples.

Indications for the preparations according to the invention are, for example, flatulence, a feeling of repletion, abdominal rumbling, pains caused by pressure, spastic pains.

The tablets are highly compatible. In particular, they are distinguished by their outstanding influence on spastic pain conditions and on the frequently persistent pains caused by pressure.

For digestion, enzymes are normally formed primarily by the pancreas, degrading food into a form in which it can be used by the body. The preparation according to the invention, which is fat-splitting by virtue of its lipase content, protein-splitting by virtue of its protease content and starch-degrading

by virtue of its amylase content, thus replaces a deficit of these enzymes.

In addition, the digestion of fats improved by the lipase favourably influences a disturbed liver and gall bladder function.

The enzyme combination is fully effective both in the acid range and also in the alkaline range of the digestive tract and thus enables food to be optimally digested.

The preparation according to the invention has the advantage of the immediate availability of the fully active enzymes. The effect actually occurs in the stomach itself after admixture with the food pulp without any delays attributable to decomposition or dissolution times.

The invention is further illustrated by the following Examples.

EXAMPLE 2

One example of a firmly formed stabilised enzyme preparation is a chewing tablet which consists of the following components:

	parts
Fungus lipase from *Rhizopus arrhizus*	20 to 40
Enzyme concentrate from *Aspergillus oryzae*	100 to 150
Dialkyl polysiloxane which may contain up to at most 10% of its weight of silica gel	50 to 275
Active alumina	250 to 750
Sweet whey powder	250 to 1000
Saccharose	250 to 750
Saccharin sodium	1 to 5
Sorbitol	100 to 200
Highly disperse silica	30 to 70
Talcum	25 to 75
Standard pharmaceutical flavour correctants (for example caramel aroma, vanillin)	5 to 20

Particularly suitable dialkyl polysiloxanes are the dialkyl polysiloxanes described in German Offenlegungsschrift No. 2,408,290, page 1, last paragraph and page 2.

The dialkyl polysiloxane may be activated by 0.5 to 10% by weight and preferably by 5 to 10% by weight (based on the quantity of dialkyl polysiloxane) of silica gel or silicon dioxide. The silicon dioxide described on page 3 of German Offenlegungsschrift No. 2,408,290 is particularly suitable for this purpose.

The chewing tablet is produced for example as follows:

I. Granulate

1) 20.0 kg of saccharose and 40.0 kg of dried aluminium hydroxide gel are sieved (mesh width approximately 1.2 mm) and mixed in a suitable mixer (forced circulation mixer, for example a Diosna, the word "Diosna" being a Registered Trade Mark mixer).

= Mixture I/1

2) 21.04 kg of dimethyl polysiloxane activated with silica gel are added to and intensively mixed with mixture I/1.

= Mixture I/2

3) Granulation

20.0 kg of saccharose are dissolved while stirring at +80°C in 10.0 kg of demineralised water.

= Solution 1

0.2 kg of saccharin sodium are dissolved while stirring in 0.8 kg of demineralised water.

= Solution 2

Solution 1 is allowed to cool to room temperature before further processing. Solution 2 is then introduced while stirring into solution 1.

= Sugar solution

Mixture I/2 is moistened with the sugar solution and intensively compounded in a suitable mixer (for example Diosna).

Approximately 0.8 kg of demineralised water are then added. The quantity of water and mixing time must be such that a uniformly moistened mass is formed. The moist mass is passed through a granulating machine (3 to 4 mm) and dried at 60 to 65°C.

The dry coarse-grained mass is passed through a 1.2 mm mesh sieve.

= Granulate for the enzyme chewing tablets

Maximum relative moisture content of the dried granulate: 10%.

II. Production of the enzyme pressing composition for 80,000 tablets

1) 0.84 kg of talcum
0.04 kg of highly disperse silica
0.20 kg of vanillin
0.60 kg of caramel aroma
1.68 kg

are sieved (mesh width 0.5 mm) and subsequently mixed in a suitable mixer.

= Mixture II/1

2) 60.0 kg of sweet whey powder
4.0 kg of talcum
3.48 kg of highly disperse silica
67.48 kg

are mixed in a tumble mixer (for example Rotex) and passed through an Express sieve (mesh width 1.0 mm).

= Mixture II/2

3) 1.6 kg of fungus lipase from *Rhizopus arrhizus*
9.2 kg of enzyme concentrate from *Aspergillus oryzae*
1.68 kg of mixture II/1

12.48 kg

are combined with approximately 10 kg of mixture II/2 and passed through an express sieve (mesh width 1.0 mm). About another 5 kg of mixture II/2 are passed through the express sieve used.

=Mixtures II/3=27.48 kg

4) 27.48 kg of mixture II/3
52.48 kg of mixture II/2 (=residue)

79.96 kg

are mixed in a suitable mixer (for example Diosna).

=Mixture II/4

5) 10.96 kg of sorbitol are passed through a 1.2 mm mesh sieve and dried for about 5 hours at 60°C. Relative moisture content of the dried sorbitol: 15% (±5%).

6) 79.96 kg of mixture II/4
10.8 kg of dried sorbitol
101.24 kg of granulate for enzyme chewing tablets

192.00 kg

are mixed in a suitable mixer (for example Turbula; Turbula drum approx. 370 litres capacity, 1 hour at 10 rpm).

=Composition ready for pressing

Maximum relative moisture content of the pressing composition: 22%. Bulk factor: 100 g=approx. 140 ml.

III. Pressing operation

Tablets weighing 2.4 g are produced from the pressing composition in an eccentric or rotary press.

The following tablet formulations are made up in accordance with Example 2:

EXAMPLE 3

Tablet formulation

Lipase S 60 from *Rhizopus arrhizus*	20 mg
Sweet whey powder	850 mg
Saccharose	528 mg
Saccharin sodium	2.5 mg
Sorbitol	135 mg
Highly disperse silica	44 mg
Talcum	60.5 mg
Vanillin	2.5 mg
Caramel aroma	7.5 mg
	1650.0 mg

EXAMPLE 4

Tablet formulation

Lipase S 60 from *Rhizopus arrhizus*	20 mg
Dimethyl polysiloxane	250 mg
Activated with silica gel	13 mg
Sweet whey powder	850 mg
Saccharose	515 mg
Saccharin sodium	2.5 mg
Sorbitol	135 mg
Highly disperse silica	44 mg
Talcum	60.5 mg
Vanillin	2.5 mg
Caramel aroma	7.5 mg
	1900.0 mg

EXAMPLE 5

Tablet formulation

Lipase S 60 from *Rhizopus arrhizus*	20 mg
Dimethyl polysiloxane	250 mg
Activated with silica gel	13 mg
Aluminium hydroxide gel	500 mg
Sweet whey powder	850 mg
Saccharose	515 mg
Saccharin sodium	2.5 mg
Sorbitol	135 mg
Highly disperse silica	44 mg
Talcum	60.5 mg
Vanillin	2.5 mg
Caramel aroma	7.5 mg
	2400.0 mg

EXAMPLE 6

Tablet formulation

Lipase S 60 from *Rhizopus arrhizus*	20 mg
Enzyme concentrate from *Aspergillus oryzae*	100 mg
Dimethyl polysiloxane	250 mg
Activated with silica gel	13 mg
Aluminium hydroxide gel	500 mg
Sweet whey powder	750 mg
Saccharose	515 mg
Saccharin sodium	2.5 mg
Sorbitol	135 mg
Highly disperse silica	44 mg
Talcum	60.5 mg
Vanillin	2.5 mg
Caramel aroma	7.5 mg
	2400.0 mg

WHAT WE CLAIM IS:—

1. An enzyme preparation containing a lipase of non-animal origin and, as stabiliser, 10 to 50 parts by weight (based on 1 part by weight of lipase) of a mixture of the following constituents: 40 to 90% of lactose; 8 to 50% of whey protein; 0.1 to 7% of milk fat; 0.1 to 10% of whey minerals and 0.1 to 5% of water (%=% by weight).

2. A preparation as claimed in Claim 1,

containing as stabiliser a whey product of the following composition: 49 to 51% of lactose; 36 to 40% of whey protein; 3 to 5% of milk fat; 6 to 7% of whey minerals; 2 to 3% water.

3. An enzyme preparation containing a lipase of non-animal origin and, as stabiliser, 10 to 50 parts by weight (based on 1 part by weight of lipase) of a mixture of the following constituents: 70 to 90% of lactose; 8 to 20% of whey protein; 0.1 to 3% of milk fat; 0.1 to 5% of whey minerals and 0.1 to 3% of water (% = % by weight).

4. A preparation as claimed in Claim 3, containing sweet whey powder as stabiliser.

5. An enzyme preparation substantially as described with particular reference to Example 1.

6. A pharmaceutical preparation containing a lipase of non-animal origin and from 10 to 50 parts by weight (based on 1 part by weight of the lipase) of a mixture of the following constituents: 40 to 90% of lactose; 8 to 50% of whey protein; 0.1 to 7% of milk fat; 0.1 to 10% of whey minerals and 0.1 to 5% of water (% = % by weight).

7. A preparation as claimed in Claim 6, containing as stabiliser a whey product of the following composition: 49 to 51% of lactose; 36 to 40% of whey protein; 3 to 5% of milk fat; 6 to 7% of whey minerals; 2 to 3% of water.

8. A preparation as claimed in Claim 6 or 7 which additionally contains a proteinase and/or an amylase.

9. A preparation as claimed in Claim 8, containing an enzyme concentrate of *Aspergillus oryzae*.

10. A preparation as claimed in any of Claims 6 to 9 which is intended for oral administration.

11. A preparation as claimed in Claim 10, in the form of tablets.

12. A preparation as claimed in Claim 11, in the form of chewing tablets.

13. A pharmaceutical preparation containing a lipase of non-animal origin and from 10 to 50 parts by weight (based on 1 part of the lipase) of a mixture of the following constituents: 70 to 90% of lactose, 8 to 20% of whey protein; 0.1 to 3% of milk fat; 0.1 to 5% of whey minerals and 0.1 to 3% of water (% = % by weight).

14. A preparation as claimed in Claim 13, containing sweet whey powder as stabiliser.

15. A preparation as claimed in Claim 13 or 14 additionally containing a proteinase and/or an amylase.

16. A preparation as claimed in Claim 15, containing an enzyme concentrate from *Aspergillus oryzae*.

17. A preparation as claimed in any of Claims 13 to 16 which is intended for oral administration.

18. A preparation as claimed in Claim 17, in the form of tablets.

19. A preparation as claimed in Claim 18, in the form of chewing tablets.

20. A pharmaceutical preparation substantially as described with reference to any of Examples 2 to 6.

21. A process for the production of a lipase-containing enzyme preparation with improved activity, wherein the lipase is of non-animal origin, which comprises mixing the lipase for the purposes of stabilisation with 10 to 50 parts by weight (based on 1 part by weight of lipase) of a mixture of the following constituents: 40 to 90% of lactose; 8 to 50% of whey proteins; 0.1 to 7% of milk fat; 0.1 to 10% of whey minerals and 0.1 to 5% of water (% = % by weight).

22. A process as claimed in Claim 21, wherein a whey product of the following composition is used as stabiliser: 49 to 51% of lactose; 36 to 40% of whey protein; 3 to 5% of milk fat; 6 to 7% of whey minerals; 2 to 3% of water.

23. A process for the production of a lipase-containing enzyme preparation with improved activity, wherein the lipase is of non-animal origin which comprises mixing the lipase for the purposes of stabilisation with 10 to 50 parts by weight (based on 1 part by weight of lipase) of a mixture consisting of the following constituents: 70 to 90% of lactose; 8 to 20% of whey protein; 0.1 to 3% of milk fat; 0.1 to 5% of whey minerals and 0.1 to 3% of water (% = % by weight).

24. A process as claimed in Claim 23, wherein sweet whey powder is used as stabiliser.

25. A process for the production of a lipase-containing enzyme preparation substantially as described with particular reference to Example 1.

26. A process for the production of a pharmaceutical preparation which comprises mixing a lipase of non-animal origin with 10 to 50 parts by weight (based on 1 part by weight of the lipase) of a mixture of 40 to 90% of lactose; 8 to 50% of whey protein; 0.1 to 7% of milk fat; 0.1 to 10% of whey minerals and 0.1 to 5% of water (% = % by weight).

27. A process as claimed in Claim 26 wherein the lipase is also mixed with at least one other pharmaceutical auxiliary or additive.

28. A process as claimed in Claim 26 or 27, wherein the mixture obtained is pressed into tablets.

29. A process for the production of a pharmaceutical preparation which comprises mixing a lipase of non-animal origin with 10 to 50 parts by weight (based on 1 part by weight of the lipase) of a mixture of 70 to 90% of lactose, 8 to 20% of whey protein,

0.1 to 0.3% of milk fat, 0.1 to 5% of whey minerals and 0.1 to 3% of water (%=% by weight).

30. A process as claimed in Claim 29 wherein the lipase is also mixed with at least one other pharmaceutical auxiliary or additive.

31. A process as claimed in Claim 29 or 30, wherein the mixture obtained is pressed into tablets.

32. A process for the production of a pharmaceutical preparation substantially as described with particular reference to any of Examples 2 to 6.

33. A lipase-containing enzyme preparation when produced by a process as claimed in Claim 21 or 22.

34. A lipase-containing enzyme preparation when produced by a process as claimed in any of Claims 23 to 25.

35. A pharmaceutical preparation when produced by a process as claimed in any of Claims 26 to 28.

36. A pharmaceutical preparation when produced by a process as claimed in any of Claims 29 to 32.

37. A process for stabilising a lipase-containing enzyme preparation, the lipase being of non-animal origin, which comprises incorporating therein a mixture of 40 to 90% of lactose, 8 to 50% of whey protein, 0.1 to 7% of milk fat, 0.1 to 10% of whey minerals and 0.1 to 5% of water (%=% by weight).

38. A process as claimed in Claim 37, wherein a mixture of the following composition is used: 49 to 51% of lactose; 36 to 40% of whey protein; 3 to 5% of milk fat; 6 to 7% of whey minerals; 2 to 3% of water.

39. A process for stabilising a lipase-containing enzyme preparation wherein the lipase is of non-animal origin, which comprises incorporating therein a mixture of 70 to 90% of lactose; 8 to 20% of whey protein; 0.1 to 3% of milk fat; 0.1 to 5% of whey minerals and 0.1 to 3% of water (%=% by weight).

40. A process as claimed in Claim 39, wherein sweet whey powder is used.

41. A process for stabilising a lipase-containing enzyme preparation substantially as described with particular reference to Example 1.

42. A process for stabilising a pharmaceutical preparation containing lipase of non-animal origin which comprises incorporating therein from 10 to 50 parts by weight, based on 1 part by weight of the lipase), of a mixture of 40 to 90% of lactose, 8 to 50% of whey protein, 0.1 to 7% of milk fat, 0.1 to 10% of whey minerals and 0.1 to 5% of water (%=% by weight).

43. A process as claimed in Claim 42 wherein a whey product of the following composition is used: 49 to 51% of lactose; 36 to 40% of whey protein; 3 to 5% of milk fat; 6 to 7% of whey minerals; 2 to 3% of water.

44. A process for stabilising a pharmaceutical preparation containing lipase of non-animal origin which comprises incorporating therein from 10 to 50 parts by weight, based on 1 part by weight of the lipase, of a mixture of 70 to 90% of lactose, 8 to 20% of whey protein, 0.1 to 3% of milk fat, 0.1 to 5% of whey minerals and 0.1 to 3% of water (%=% by weight).

45. A process as claimed in Claim 44, wherein sweet whey powder is used.

46. A process for stabilising a pharmaceutical preparation substantially as described with particular reference to any of Examples 2 to 6.

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